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_PDA J Pharm Sci and Tech_ 2013, 67 376-386
Access the most recent version at doi:10.5731/pdajpst.2013.00928
A Preliminary Investigation into the Ability of Three Rapid Microbiological Methods To Detect Microorganisms in Hospital Intravenous Pharmaceuticals

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ABSTRACT: This study investigated the capability of three rapid microbiological methods to detect microorganisms in aseptically prepared pharmacy preparations at National Health Service hospitals in the United Kingdom. BacT/ALERT 3D (bioMerieux), AKuScreen (Celsis), and BactiFlow ALS (AES Chemunex) technologies were used to detect levels of microorganisms in pharmaceutical products. Four products selected to represent the range of pharmaceuticals prepared in National Health Service hospital pharmacy departments were spiked with known levels of microorganisms. The presence of microorganisms in these products was then determined using each of the rapid microbiological methods and compared to the number determined by traditional total aerobic microbial count methodology. An evaluation of the performance parameters associated with each of the methods, including cost analysis, was also undertaken. There was good correlation between rapid microbiological methods and total aerobic microbial count for heparin and parenteral nutrition products. The rapid microbiological methods had difficulty recovering Gram-positive organisms from vancomycin and methotrexate products; however, protocol developments demonstrated that this was surmountable. The main differences between the rapid microbiological method systems were time-to-result, the initial equipment cost, and the skill required to operate the instruments. The main finding from this work is that rapid microbiological methods can detect microbial contamination of hospital pharmaceutical products in a reduced time when compared to traditional microbiological techniques. The instrument comparison showed that Celsis AKuScreen provided the most rapid result for detecting bacteria; BacT/ALERT was the least expensive instrument and the simplest system to use; and BactiFlow ALS was the most expensive and more complex to use and gave intermediate time to results.

KEYWORDS: BacT/ALERT 3D, AKuScreen, BactiFlow ALS, Microbial detection, Aseptic manufacture, Quality, Rapid

LAY ABSTRACT: Traditional methods to assure quality and detect any microbial contaminants in intravenous products takes at least 2 weeks to complete. Hospital-prepared intravenous products are often high risk and have short shelf lives. This sometimes means that the traditional quality microbiological results are only available after the product has been administered to the patient. There have been some fatal incidents in which contaminated intravenous products have been used. Rapid microbiological methods have been used in the food, cosmetic, and pharmaceutical industries; however, they have not as yet been used in United Kingdom National Health Service pharmacy manufacturing. Rapid microbiological methods are able to detect contaminants in shortened periods of time. This study investigates the ability of three rapid microbiological methods to detect artificially contaminated, National Health Service–prepared intravenous products and compares the results to those obtained from traditional total aerobic microbial count methodology. The results show that there are differences between the methods; however, generally rapid microbiological methods are able to detect contaminants from pharmacy-prepared intravenous products quicker than current traditional methods.
Introduction

The majority of medicines used within the National Health Service (NHS) in the United Kingdom (UK) are products with a full Marketing Authorisation from the statutory regulatory body, the Medicines and Healthcare Products Regulatory Agency (MHRA), and are manufactured by commercial pharmaceutical companies. However, there are a significant number of clinical situations where there is no licensed product available or where the licensed product is not in a suitable pharmaceutical form. These medicines are nevertheless an essential part of clinical practice. These are often prepared in hospital pharmacies as unlicensed medicines. There are several categories of regulation for unlicensed aseptic preparation processes, including those regulated by the UK Statutory body (MHRA) for bulk manufacture (pharmaceutical specials) and those regulated by the UK professional body the General Pharmaceutical Council (GPhC) for single-patient preparation (prepared under the Section 10 exemption of the Medicines Act 1968). There may be some similarities to be drawn with United States (US) terminology of compounding and bulk compounding.

In UK law there is no requirement for a sterility test to be performed before administration of unlicensed aseptic products with expiry dates of less than 3 months; however, adequate good manufacturing practice (GMP) and quality assurance (QA) must still be ensured (1–3). These tests will include quantification of viable microorganisms in the manufacturing environment and nutrient broth simulations carried out at the end of production. If time permits, units may decide to carry out viability counts or sterility testing; however, this is not a UK legal requirement.

In the US traditional compounding pharmacies are not registered with the Food and Drug Administration and are regulated by state law (state boards of pharmacy and state departments of health). Regulatory guidelines for sterile compounding exist in the USP (Chapter <797> Pharmaceutical Compounding—Sterile Preparations); however, the level of adherence to and regulation of this in respect to compounding and bulk compounding is variable and dependant on individual state law.

Traditional microbiological techniques can take considerable time to a result, as they are growth-based and require either visible colony formation on an agar plate or turbidity of broth samples. For example, a sterility test will take at least 14 days to complete. However, many aseptic products produced in hospitals are inherently chemically unstable and have short shelf lives. These shelf lives can often be less than 28 days and sometimes as short as 24 h. These products are often produced for immuno-compromised patients, such as neonates and those on chemotherapy. Due to short shelf lives, quality control tests upon these products are limited and release can be based on retrospective GMP assessments alone. These compromised testing approaches are not without problems, and there are examples of system failures. In Manchester three children died after receiving contaminated parenteral nutrition (PN) (4), and there have been reports of PN associated with outbreaks of Serratia odorifera (5). More recently, three babies died from septicaemia caused by Enterobacter cloacae–contaminated PN administered in a hospital in Mainz, Germany (6).

A more rapid method of microbiological assessment could improve the QA of these products. Rapid microbiological methods (RMMs) have been used for many years in food and clinical microbiology, and more recently they have been explored for inclusion into commercial pharmaceutical manufacturing processes (7–12). Detection of microorganisms with these RMMs can occur within hours rather than days or weeks. The British, European, and United States Pharmacopoeia now contain sections on RMMs (13–15). However, RMMs have yet to become part of the routine quality control assessment of short shelf life, unlicensed pharmaceuticals prepared within hospital pharmacies. There is limited peer-reviewed work in this area. This study seeks to assess the possibilities of using these techniques to advance the microbiological quality control assessment of short-shelf life, aseptically prepared, unlicensed products.

Objectives

Here, we report on the evaluation of three different RMM systems for their ability to improve QA processes associated with four aseptic intravenous (IV) medicines manufactured by NHS Pharmacy Wales.

Materials and Methods

RMM Systems

Three commercial RMM systems were chosen for evaluation based on different detection principles: (1) BacT/ALERT 3D (bioMérieux, Basingstoke, UK) based on colour changes associated with carbon dioxide generated by metabolising microorganisms, (2)
AKuScreen (Celsis, Cambridge, UK) based on microbial adenylate kinase (AK)-enhanced adenosine triphosphate (ATP) bioluminescence, and (3) BactiFlow ALS (AES Chemunex, Basingstoke, UK) based on direct fluorescent labelling. The RMM systems were kindly provided by the respective companies together with appropriate training.

Microbiological Validation Overview

Four aseptically prepared products were identified as representative of NHS special manufacture. Initial feasibility studies were performed by each vendor and it was determined that product interference was minimal or did not exist with the procedures used in the current study. A spiking study was then undertaken whereby four microorganisms were spiked at low inoculum into the four products and traditional methods of microbial recovery, as described in the British Pharmacopoeia (16), were compared with results from the three RMMs. We were investigating the use of RMM primarily as an in-process control with aseptic products and therefore used challenge organisms that were more frequently seen in our manufacturing environment rather than those of compendial challenge requirements.

The four aseptic products used were Heparin 100 IU/mL, neonatal 7.5% glucose PN, vancomycin intrathecal injection (10 mg/2 mL), and methotrexate injection (15 mg/0.6 mL). These products were compounded to GMP standards in the NHS Pharmacy manufacturing department of the Cardiff and Vale University Health Board and were assigned short shelf lives given the requirement to manipulate the licensed product and/or supply in ready-to-use formats.

The four microorganisms investigated were *Staphylococcus aureus* (NCIMB 9518), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), and spores of *Bacillus subtilis* (ATCC 6051). All bacteria were subcultured from tryptone soya agar (TSA, Oxoid, Basingstoke, UK) slopes, incubated at 30 °C, and plated regularly to check purity according to a standard protocol (EN 1040) (17). Working cultures were prepared from TSA slopes by resuspension/centrifugation in tryptone sodium chloride (TSC, containing 1 g/L tryptone and 8.5 g/L NaCl). The concentration of bacterial culture was adjusted to $1 \times 10^8$ cfu/mL using a standard calibration graph of OD$_{590}$ against bacterial viable count.

*C. albicans* was subcultured on sabouraud dextrose agar (SDA, Oxoid). Working inocula were prepared from SDA slopes and diluted in tryptone sodium chloride (TSC). SDA slopes were incubated at 25 °C. The preparation of *B. subtilis* spore suspension was based on the European standard EN14347 (18). Homogeneity of the spore suspension was checked using standard malachite green staining. The spore suspension was adjusted to $1 \times 10^8$ spores/mL following total aerobic microbial count (TAMC) enumeration. The spore suspension was maintained at 4 °C.

The drop count (Miles-Misra) method (19) was validated and subsequently used for stock enumerations.

Neutralisation Study

It was necessary to neutralise any possible antimicrobial activity arising from each pharmaceutical product to prevent any false negatives. Based on a series of preliminary experiments and the diverse and challenging range of products, dilution-filtration was selected. Validation of neutralisation by dilution-filtration was carried out as follows: 1 mL of product was mixed with 99 mL buffered peptone solution (BPS, bioMerieux), 50 μL of microbial inoculum ($1 \times 10^3$ cfu/mL) was then added, vortexed, and filtered through a 0.45 μm membrane filter (Biosart 100, Sartorius, Epsom, UK). The membrane filter was washed with 100 mL BPS and then plated on TSA at 30 °C (bacteria) and SDA at 25 °C (yeast) and incubated for 24 h and 48 h, respectively. Colonies formed, being indicative of surviving bacteria/yeast, were then enumerated. A product will have been neutralised if the test organism does not differ by a value of $\geq 1$ log reduction from the control.

Microbial Inactivation Kinetics

It is anticipated that any products manufactured in a licensed unit will be sterile or have low bioburden levels; however, there have been examples of contamination, as previously described. The aim of this study was to compare traditional and rapid microbial detection methods when typical scenarios were modelled. It was therefore necessary to determine initial microbial inoculum concentrations to add to each product that would result in a suitably low residual microbial concentration ($\sim 10$ cfu/mL) after a fixed contact time, taking into account the possible toxicity of the product. Preliminary studies (20) of microbial survival in
these products were used to determine the initial spiking inoculum with the precise inoculum size at time of testing being confirmed by simultaneous sampling and enumeration by TAMC.

**Spiking Study**

Microbial inocula from agar slopes were washed with TSC and re-suspended to $1 \times 10^8 \text{cfu/mL in TSC}$. The initial spiking concentrations varied depending on the survival kinetics of the organisms and products being tested. Five millilitres of manufactured products were dispensed into 20 mL sterile glass universal vials. Each product was then spiked with 50 $\mu$L of an appropriate inoculum concentration of each of the four microorganisms or 50 $\mu$L of TSC (a negative control) then left in contact for exactly 10 min. One millilitre was then removed for each of the enumeration tests of TAMC and the three RMMs. There was less than 1 min between taking each of the split samples.

**Enumeration of Surviving Bacteria in Products**

**Traditional TAMC:** Drawing on the methodology of the BP, each 1 mL of spiked product was diluted into 99 mL of PBS and passed through a 0.45 $\mu$m membrane filter (Biosart® 100, Sartorius). Membranes were washed (100 mL TSC) then plated on TSA at 30°C (bacteria) and SDA at 25 °C (yeast) and incubated for 24 h and 48 h, respectively. Colonies formed, being indicative of surviving bacteria/yeast, were then enumerated. This enumeration data, however, was only used as a presence/absence parameter when the results were compared with the responses from the three qualitative RMMs.

**RMMs:** BacT/ALERT (bioMérieux): Each 1 mL of spiked product was inoculated into a dedicated BacT/ALERT broth vial (40 mL i AST) and placed immediately onto the BacT/ALERT system. The BacT/ALERT culture bottle was incubated at 30 °C and scanned every 10 min for colour change resulting from CO$_2$ production. CO$_2$ changes the colour of the liquid emulsion sensor found in each bottle, from light to dark. The light reflected is continuously monitored by a solid-state photodetector. If there is high initial CO$_2$ content, an unusually high rate of CO$_2$ production, and/or a sustained rate of CO$_2$, the sample is determined positive. If the CO$_2$ level does not change significantly after a user-specified period of time, which was 10 days for this trial, the sample was determined to be negative. The instrument’s audible and visual alarms immediately informed the user when a bottle shows positive for microbial growth.

AKuScreen (Celsis): Each 1 mL of spiked product was added to 99 mL of tryptone soya broth then placed on a shaking incubator at 30 °C for 18 h. This pre-incubation process allows low levels of organisms to be amplified to above the level of detection for this system. AKuScreen employs an automated assay that uses microbial AK to convert adenosine diphosphate (provided to the assay) into ATP, which is then detected using a bioluminescence assay. Preparation of the system requires a start-up routine of cleaning and daily controls (according to company algorithms) and a shut-down clean. Daily controls include the measurement of background levels of ATP in the broth used for the product enrichment process. Typically three times this background level is set as the threshold for determining a positive sample for the batch, although alternate approaches to establishing the cut-off may be utilised. Results are presented as relative light units (RLU) and interpreted as a positive or negative outcome normalised against the daily controls.

BactiFlow ALS (AES Chemunex): Each 1 mL of spiked product was added to 99 mL of tryptone soya broth then placed on a shaking incubator at 30 °C for 24 h. This pre-incubation process allows low levels of organisms to be amplified to above the level of detection for this system. BactiFlow ALS uses an auto-analysers and a laser beam to detect microorganisms. Preparation of the system consists of a cleaning and calibration run together with database labelling of samples. Fifty microlitre samples are loaded onto the BactiFlow ALS in batches of 25 and automatically assayed. The results are recorded on associated software. At the end of the session, a clean-down is also required. Background noise levels of fluorescence were determined during the protocol development phase and threshold levels set for each product for the duration of this study. Results are presented as fluorescence counts per millilitre and interpreted as a positive or negative.

**Performance Parameters**

A table of performance characteristics was drawn up based on the user specification and other parameters of interest such as ease of use and time to results (Table I). Data from preliminary studies was then gathered to-
Return on Investment

A cost evaluation exercise was carried out using a batch (50) of 300 mL parenteral nutrition 7.5% glucose (PN) bags. Guidance QA of this product currently consists of broth runs on 10% of the batch (5/100 filled broth bags). These are incubated for 7 days and inspected for turbidity before batch release and subsequently incubated for a total of 14 days, providing retrospective QA data. The rapid microbiology techniques have been priced according to the following model: capital equipment, maintenance fees, consumables, facilities, and staff time. Figures related to price of the equipment, length of life, and maintenance costs have been provided by each company. Prices have been normalised using the assumption that the manufacturing unit produces 250 batches of products per year.

The protocols provided by the companies for this study suggested product volume(s) to be tested per vial, for example, each bioMérieux test vial can accommodate a volume up to 10 mL and both Celsis and AES used 1 mL product volume inoculated into 99 mL of broth for the overnight incubation phase. If sterility test guidelines were followed for the test batch of 50 PN 300 mL bags, 30 mL from each of five bags would need to be tested. Using the current protocols, large numbers of test samples and consumables from each company would be required to test this batch of PN. However, in practice protocols would probably be developed either to reduce the sampling volume by a pre-filtration step or to accommodate the larger sampling volumes, altering staff skill, time, and consumable requirements. The technologies examined in this study vary in their ability to accommodate larger volumes and different incubation broths. For this costing exercise, however, we have normalised the sampling volume to 1 mL per unit tested, which can be accom-

### TABLE I

<table>
<thead>
<tr>
<th>RMM</th>
<th>Trade name</th>
<th>Company</th>
<th>Principle</th>
<th>Detection system</th>
<th>Detection type</th>
<th>Particular</th>
<th>Limit of detection</th>
<th>Sample volume</th>
<th>Capacity</th>
<th>Time at which results are read**</th>
<th>Ease of use</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BactiFlow ALS</td>
<td>AES Chemunex</td>
<td>Fluorescent labelling</td>
<td>Flow cytometry</td>
<td>Qualitative</td>
<td></td>
<td>1 cell per g(^+^)</td>
<td>pre-incubation step (1 in 100 dilution) then 50 μL samples</td>
<td>~200/day</td>
<td>24 h</td>
<td>Medium</td>
<td>£90K</td>
</tr>
<tr>
<td></td>
<td>BacT/ALERT</td>
<td>bioMérieux</td>
<td>Growth based</td>
<td>CO(_2)/Colour change</td>
<td>Qualitative</td>
<td></td>
<td>1 cell per sample</td>
<td>Up to 10 mL per 40 mL vial</td>
<td>~50/day</td>
<td>24–72 h</td>
<td>Easy</td>
<td>£30K</td>
</tr>
<tr>
<td></td>
<td>AKuScreen</td>
<td>Celsis</td>
<td>ATP Bioluminescence</td>
<td>RLU/luminosity</td>
<td>Qualitative</td>
<td></td>
<td>1 cell per g(^+^)</td>
<td>pre-incubation step (1 in 100 dilution) then 50 μL samples</td>
<td>~1000/day</td>
<td>18 h</td>
<td>Medium</td>
<td>£67K</td>
</tr>
</tbody>
</table>

* as of November 2010.
** this was user selected and should not be confused with detection times.
^ only after an enrichment phase.
modated by all the current protocols. Facility costs relate to the environmental quality of QA rooms required to manipulate samples post-manufacture. Traditional sterility testing of terminally sterilised products requires a Grade A QA facility for sample manipulations (1). Traditional broth testing of aseptic products only requires incubation in an unclassified room. Facilities relating to sample manipulation in RMM testing depend on how initial samples are taken and what manipulations are subsequently required.

After consideration of each company’s basic protocol, it was determined that if product samples can be inoculated into the pre-enrichment broths in the Grade A environment, then any further sample manipulations can be carried out in unclassified facilities. bioMérieux samples did not need to be manipulated further and were simply placed onto the BacT/ALERT system. Celsis and AES Chemunex samples needed to be further manipulated after the desired pre-incubation period. However, any contamination at this subsequent point would not reach the threshold levels for both systems and therefore any positive result is representative of contamination from the original sample. Staff time involved is also representative of the sample manipulations required, including time and complexity involved. bioMérieux requires basic and minimal input, whereas Celsis and AES Chemunex require more complex and prolonged input from staff. A contract sterility test price has been added for comparison, although this is not carried out in practice on the PN product used in this evaluation. There are other factors that could be taken into consideration; these are highlighted in various publications where more detailed analytical approaches are taken to return on investment (21, 22).

Results

User Specification

A user specification list was developed based on QA requirements relating to short-dated and high-risk aseptically manufactured products. A number of criteria important for the end-users of RMM are as follows: (1) results available within 24–36 h, (2) detection limit comparable with previous methods, (3) good reproducibility, (4) minimal sample manipulation, (5) equipment usable across a wide range of products, (6) no interference from active or excipients in products, and (7) potential for minimal operator demands/train-

ing requirements. The user specification list was used to assess the performance and return on investment for each RMM (Table I).

Microbial Validation

All products passed the feasibility tests with each RMM technology.

Data from the neutralisation study demonstrated that all product/microorganism combinations passed our study criteria. PN and heparin showed little evidence of reduction in numbers of microorganisms. With vancomycin and methotrexate, however, some microbial death was noted but study criteria were still met. Inactivation kinetic studies allowed initial spiking concentration of each of the microorganisms to be determined for each product.

Spiking Study

The results from each RMM are presented together in Table II as concordance patterns between the RMM and TAMC, with data shown for each triplicate repetition performed. The spiking study allowed detection rates to be examined, with AES Chemunex and Celsis systems being read at 24 h and 18 h, respectively. BacT/ALERT bioMérieux followed a generic protocol that initially suggested reading results at 3 days (although time to positive results were collected over a 10 day period). All negative controls showed no growth. There was complete concordance between all RMMs (generic protocols) and the TAMC with heparin and PN. The recovery of Gram-positive organisms from vancomycin and methotrexate proved more problematic for the RMM, however; BacT/ALERT was able to recover *B. subtilis* from methotrexate equivalent to traditional methods after 3 days incubation (as per protocol). Further investigations of daily results with the BacT/ALERT (bioMérieux) over a 10 day period are presented as concordance patterns in Table III. The results show that with further validation this system could potentially be used to provide more rapid results for certain products. For example all the microorganisms tested with this system, except *Candida* spp., were detected in PN and heparin within 24 h. It is to be noted, however, that the incubation temperature used was 30 °C, which is not optimal for yeast species and at which a lower temperature incubation may allow the full
range of organisms to be recovered within the 24 h period (not evaluated).

**Return on Investment**

Table IV shows the results of a cost evaluation exercise based on current RMM procedures compared with traditional QA tests. The cost evaluation exercise was carried out using a batch (50) of 300 mL PN bags and provides estimated values. The costs for a sterility test and an aseptic broth run are presented as contract prices for comparison. The running costs of the three systems are very similar. The main difference is in the capital costs and the staff skill sets needed to operate the different systems. While the bioMérieux technology was relatively simple to use, both the Celsis and AES Chemunex systems required a more complex set of operator skills.

**Discussion**

The modernisation and rationalisation agendas (23) across the UK have facilitated NHS manufacturing

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**TABLE II**

Concordance Patterns

<table>
<thead>
<tr>
<th>Product</th>
<th>Microbe</th>
<th>AES Chemunex (24 h)*</th>
<th>bioMérieux (3 days)</th>
<th>Celsis (18 h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>run</td>
<td>run</td>
<td>run</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1  2  3</td>
<td>1  2  3</td>
<td>1  2  3</td>
</tr>
<tr>
<td>Heparin</td>
<td>S. aureus</td>
<td><strong>+</strong></td>
<td><strong>+</strong></td>
<td><strong>+</strong></td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa</td>
<td><strong>+</strong></td>
<td><strong>+</strong></td>
<td><strong>+</strong></td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td><strong>+</strong></td>
<td><strong>+</strong></td>
<td><strong>+</strong></td>
</tr>
<tr>
<td></td>
<td>B. subtilis (spore)</td>
<td><strong>+</strong></td>
<td><strong>+</strong></td>
<td><strong>+</strong></td>
</tr>
<tr>
<td>PN</td>
<td>S. aureus</td>
<td>++</td>
<td>++</td>
<td><strong>+</strong></td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>B. subtilis (spore)</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Vanc</td>
<td>S. aureus</td>
<td>++</td>
<td>++</td>
<td><strong>+</strong></td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>B. subtilis (spore)</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>MTX</td>
<td>S. aureus</td>
<td>++</td>
<td>++</td>
<td><strong>+</strong></td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>B. subtilis (spore)</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

* inclusive of pre-enrichment step

- **RMM and TAMC in agreement**
- **No growth with either method**
- ++ **Growth with both methods (TAMC low) 10^-100 cfu/ml**
- +++ **Growth with both methods (TAMC medium) 10^3-10^5 cfu/ml**
- ++++ **Growth with both methods (TAMC high) 10^7 – 10^9 cfu/ml**

- RMM returning a negative result while TAMC returning growth
units to expand and centralise production activities. Automation of aseptic processes is also a development being considered in future planning (24). These developments, together with national recommendations for pharmacy to increasingly control products for which preparation at ward level and/or administration is complex (25–27), heightens the need for enhanced and rapid QA activities.

This exploratory work has examined and demonstrated significant potential for the application of RMM technologies as in-process controls to the manufacture and QA of selected NHS unlicensed aseptic products. If other applications such as sterility testing and other organisms were to be used then performance, operational costs, and choice of RMM might need to be revisited.

Feasibility studies carried out with each system on the four chosen products showed that there was no interference from active or excipients.

To provide an overview of the performance of different systems compared to a traditional viability recovery method, results of the spiking experiments with the three RMMs were compiled to represent concordance.
with traditional methods (Table II). The difficulties in recovering microbes using the supplied RMM protocols from vancomycin and methotrexate could be explained by “carry over” of the biocidal products in the overnight broth incubations. Vancomycin acts on microbes by irreversibly binding to cell wall components and preventing cell wall growth, thereby preventing recovery and multiplication of any surviving microbes. Methotrexate is not employed as an antimicrobial agent but does appear to exercise an effect, which may be reversible under prolonged incubation. The three companies were given the opportunity to carry out in-house protocol development studies to address issues with the two problematic products. At the time of printing, Celsis and AES Chemunex had claimed that a pre-filtration and washing step, prior to overnight incubation of spiked product sample, was adequate to allow full concordance of this RMM with TAMC. AES Chemunex and Celsis used a closed system for doing this pre-filtration step. bioMérieux were investigating Fastidious Antibiotic Neutralisation (FAN®/H23041) bottles which contain charcoal as an adsorbant neutraliser within the dedicated broth vial. Protocol development is therefore a possibility for these problematic compounds.

When considering, more generally, the sample manipulations required and the operation of these technologies by unskilled staff, the bioMérieux system appears to be the simplest. For this system, product samples are added to dedicated broth vials that are then manually scanned and placed onto the system. Readings are then taken automatically over a pre-determined time period. Both of the other systems, however, require inoculating the product sample into broths (incubation for 18–24 h) with subsequent post-enrichment sample taking and manipulation before results can be read. This post-enrichment manipulation does require extra staff time and the attainment of additional skills. In designing a protocol, it has to be acknowledged that any increase in manipulations adds to the increased risk of adventitious contamination. The BacT/ALERT system generally showed a longer time to positive results with yeast isolates than bacteria. The BacT/ALERT system incubates at 30 °C, which may not be optimal for this yeast species; however, a recent commercial addition of the Dual T System allows incubation at both 30 °C and 25 °C.

Financial considerations were evaluated (Table IV). Contract prices have been presented for both sterility testing and broth runs. Both of these approaches, however, carry a considerable time delay in obtaining the results and must be considered alongside the estimated cost of employing these new and rapid techniques. With short expiry–dated, unlicensed products, the possibility of obtaining contamination information prior to use brings with it quality improvements compared with current quality systems, which are retrospective and limited. However, early release of longer shelf life–quarantined products may also bring quality and cost advantages.

### TABLE IV
**Cost Evaluation per Batch of 50 PN Bags**

<table>
<thead>
<tr>
<th>Cost (assumptions)</th>
<th>Traditional Sterility test</th>
<th>Traditional Aseptic broth run</th>
<th>AES Chemunex</th>
<th>bioMérieux</th>
<th>Celsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment (capital costs/yr/batch)</td>
<td>£40</td>
<td>£38</td>
<td>£38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maintenance (fee/yr/batch)</td>
<td>£22</td>
<td>£8</td>
<td>£11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consumables*</td>
<td>£25</td>
<td>£20</td>
<td>£25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facilities</td>
<td>£1</td>
<td>£1</td>
<td>£1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staff</td>
<td>£34‡</td>
<td>£34‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>£70</strong></td>
<td><strong>£75</strong></td>
<td><strong>£122</strong></td>
<td><strong>£71</strong></td>
<td><strong>£109</strong></td>
</tr>
</tbody>
</table>

* 1 mL sample volume only.

‡ Celsis and AES Chemunex Staff grade Band 5 for 2 h.

Prices as of November 2010.
General user specifications were broadly met by all three systems. The performance parameters (Table I) showed that a variety of factors need to be taken into consideration when discussing which system may be suitable for use. Sample size and capacity of the RMM may be an issue, particularly when considering pharmacopoeial sampling volumes (28) for large volume and batch products. The generic protocols used in this study indicated a limit of 10 mL per vial (bioMérieux) and a suggested maximum of 1 mL to 99 mL of broth for the other two systems. Sample volumes larger than these limits will require consideration to be given to protocol development with associated cost implications. In the return on investment exercise (Table IV), this aspect could not be addressed and a standard small volume was used to give an indication of costs. The RMM systems chosen in this study are all growth-based, where low levels of organisms are amplified by incubation until a detection signal is sufficient to determine microbial presence. They are all primarily qualitative in nature and used as presence/absence tools. Each company claims validated protocols allow detection of 1 cfu per sample (further work to explore these claims has been carried out by this research group (29)).

For this study we were interested in the QA of unlicensed aseptic intravenous product manufacture. However, personal communications and the literature indicated that all three systems can also be used qualitatively to detect contamination in many different dosage forms. The systems can be used to meet a variety of microbial quality requirements (7, 8) such as sterility, preservative efficacy, and bioburden tests. However, there are variable threshold limits for the qualitative and quantitative capabilities of each system that will require further protocol developments and specific validation exercises per product.

Acknowledgements

The Cardiff and Vale University Health Board and Welsh Assembly Government are acknowledged for their support with this work through the Trust Small Grant scheme and a Pharmacy Practice Development Scheme, respectively. The three commercial companies involved are also thanked for equipment and technical support provided for the duration of this study.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

References


18. European Standard EN14347. Chemical Disinfectants and Antiseptics – Basic Sporicidal Activity – Test Method and Requirements (Phase 1, Step 1); European Committee for Standardization: Brussels, 2005.


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